

EFFECTS OF ALCOHOL EXTRACT FROM DEFATTED SOYBEAN MEAL ON BILE ACID LEVEL, DIGESTIVE ENZYME ACTIVITY AND NUTRIENT DIGESTIBILITY OF POMPANO (*Trachinotus blochii*)

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ABSTRACT

Defatted soybean meal (SBM), which is produced by defatting soybean with solvents, has been considered as the most cost-effective substitute for fish meal (FM) in fish diets. However, SBM contains alcohol-soluble components which may interfere with growth performance and digestive physiology of fish. This study examined the effects of alcohol extract (Ext) containing alcohol-soluble components extracted from SBM on bile acid level, digestive enzyme activity and nutrient digestibility of pompano *Trachinotus blochii*. SBM was processed and separated into Ext and ethanol-extracted SBM (ESBM), thus, ESBM was SBM without alcohol-soluble components. Four experimental diets were formulated, denoted as FMD (FM-based diet), SBMD (SBM-based diet), ESBMD (ESBM-based diet), and ESBM+ExtD (ESBM-based diet plus Ext). Each diet was fed to two groups of fish (20 fish/group, 25 g/fish) for 4 weeks. Results showed that plasma lipid components, anterior intestinal bile acid level, anterior intestinal trypsin and lipase activities, and protein and lipid apparent digestibility coefficients (ADCs) of SBMD -fed fish were similar to those of ESBM+ExtD-fed fish. These parameters were significantly lower in SBMD-fed and ESBM+ExtD-fed fish than in ESBMD-fed and FMD-fed fish ($P < 0.05$). There were no significant differences in plasma total cholesterol level, trypsin activity, and protein ADC between ESBMD and FMD groups, however, total bile acid level, lipase activity, and lipid ADC were significantly lower in ESBMD -fed fish than fish fed FMD ($P < 0.05$). These results indicated that alcohol extract containing alcohol-soluble components extracted from SBM inhibited the secretions of bile acids and pancreatic digestive enzymes in pompano fish, and these abnormalities might impair nutrient digestion and absorption. The findings of the present study suggested that removing alcohol extract and supplementation of taurine could be effective for improving nutritional quality of SBM and enhancing feed utilization and growth performance of pompano which were fed SBM-based diets.

Keywords: *Trachinotus blochii*, defatted soybean meal, digestive enzyme, fish diet.

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INTRODUCTION

Fish meal (FM), which contains a high level of protein, is commonly produced from fish like sardine, anchovy and menhaden. This feedstuff is an important protein source in the aquafeed industry, but its market price has been rising due to the increasing expansion of aquaculture and the limited availability of FM resource (Olsen & Hasan, 2012; Hua et al., 2019). Defatted soybean meal (SBM), which is produced by defatting soybean with solvents (mostly hexane), has been considered to be the most cost-effective alternative to FM because of its high protein content, relatively well-balanced amino acid profile, and reasonable price (Storebakken et al., 2000; Porter & Jones, 2003). Many researches have been conducted to evaluate effects of substituting FM with SBM in fish feeds. However, dietary inclusion of a high amount of SBM has resulted in poor growth performance and feed utilization in carnivorous fish species such as yellowtail *Seriola quinqueradiata* (Shimeno et al., 1992), red sea bream *Pagrus major* (Takagi et al., 2002), Atlantic salmon *Salmo salar* (Refstie et al., 2005), rainbow trout *Oncorhynchus mykiss* (Romarheim et al., 2008), and orange-spotted grouper *Epinephelus coioides* (Wang et al., 2017). Besides the poor growth and feed performance of fish, the high inclusion of SBM reportedly induces abnormal digestive physiology such as inferior bile acid level and low digestive enzyme activities (Yamamoto et al., 2010; Nguyen et al., 2013; Murashita et al., 2018). It is possible that these disorders in digestive physiology are responsible for the low nutrient digestibility and growth performance in SBM-fed fish (Romarheim et al., 2008; Yamamoto et al., 2007; Iwashita et al., 2008).

SBM contains alcohol-soluble components not removed during oil extraction of soybean. These components have been known to cause adverse changes in digestive physiology of some carnivorous fish species which were fed SBM-containing diets. Feeding Atlantic salmon soybean molasses, which mainly contain oligosaccharides

produced by alcohol extraction, resulted in low lipid digestibility and growth performance (Olli & Krogdahl, 1995; Sorensen et al., 2011). Rainbow trouts which were fed a casein-based diet with soya saponin, soya lectin, and alcohol extract of SBM showed a decrease in bile acid level and lipid digestibility (Yamamoto et al., 2008). We previously found that alcohol extract of SBM also reduced lipid digestion and absorption in yellowtail fish (Nguyen et al., 2017). Therefore, it is possible that alcohol extract containing alcohol-soluble components from SBM is the major factor inducing impairments in carnivorous fish fed with SBM.

Trachinotus blochii (Lacepède, 1801), also known as snubnose pompano is a carnivorous marine fish species, distributed mainly in the Indo-Pacific region (Kapoor et al., 2002). It is one of the most preferred high value species for mariculture due to its fast growth rate, good meat quality and high market demand (Othman, 2008). To date, there have been no studies to investigate the effects of alcohol extract from SBM on this fish species. Therefore, to increase the feasibility of using SBM in pompano feeds, the present study aimed to evaluate the effects of alcohol extract from SBM on bile acid level, digestive enzyme activity and nutrient digestibility of pompano fish.

MATERIALS AND METHODS

Alcohol extract preparation

Defatted soybean meal [dehulled SBM; crude protein (CP) 48%] was extracted with 70, 80 and 90% aqueous ethanol at a ratio of 1:3 (w/v), starting from lower to higher concentrations. At each extraction, SBM was blended manually with the aqueous solution for 2 hours, and left at room temperature for 24 hours. The supernatant was separated from the residue by decanting and then evaporated to produce an ethanol extract (Ext, dry matter 20%), containing mainly alcohol-soluble components from SBM. The remaining residue was extracted twice in the same way as described above to produce ethanol-

extracted SBM (ESBM; CP 60%), thus, ESBM was SBM without alcohol-soluble components. The Ext was then used for supplementation to experimental diets.

Experimental diets

Four isonitrogenous and isolipidic diets were formulated with FM, ESBM, and SBM as main dietary protein sources (Table 1). The diets were denoted as follows: FMD (FM-based diet), SBMD (SBM-based diet), ESBMD (ESBM-based diet), and ESBM+ExtD (ESBM-based diet plus Ext). To make ESBM+ExtD, Ext was added to the

ESBM-based diet at a ratio that corresponds to its inclusion level in the SBM-based diet (50 g/100 g diet). Chromium oxide (5 g/kg diet) was added in all the experimental diets as a marker to estimate nutrient apparent digestibility coefficient (ADC), which presents percentage of each dietary nutrient digested and absorbed through digestion process. After the powdered ingredients were thoroughly mixed with pollock liver oil, water was added to produce a stiff dough. The dough was then pelleted using a laboratory pellet mill and stored at -20 °C until use.

Table 1. Formulation and proximate composition of the experimental diets

Ingredients (g/kg)	FMD	SBMD	ESBMD	ESBM+ExtD
Fish meal (FM)	700	330	330	330
Defatted soybean meal (SBM)	0	500	0	0
Ethanol-extracted SBM (ESBM)	0	0	400	400
Wheat flour	100	50	50	50
Pollock liver oil	60	95	95	95
Cellulose	120	5	105	5
Vitamin and mineral mixture ¹	15	15	15	15
Chromium oxide	5	5	5	5
Ethanol extract (Ext)	0	0	0	100
Proximate composition (dry matter basis, g/kg)				
Crude protein	466	464	465	468
Crude lipid	125	128	126	127
Ash	132	101	106	102

Notes: ¹Vitamin and mineral mixture (IU or mg/kg mixture): Thiamine HNO₃, 1030; riboflavin, 3070; pyridoxine HCl, 1390; cyanocobalamin, 8.1; vitamin C (L-ascorbate-2-monophosphate), 18100; vitamin A acetate, 485000; vitamin D₃ (cholecalciferol), 172000; vitamin E (DL- α -tocopherol acetate, 7010; vitamin K₃ (menadione sodium bisulfite), 1850; folic acid, 550; nicotinamide, 5200; D-calcium pantothenate, 4250; D-biotin, 16.5; inositol, 15400; ZnSO₄, 2700; MnSO₄, 1730; CuSO₄, 1310; FeSO₄, 6250; CoSO₄, 156; potassium iodide, 175; sodium selenate, 38.1.

Fish husbandry

The experiment was carried out at The National Broodstock Center for Mariculture Species, Research Institute for Aquaculture No.1 (Cat Ba, Hai Phong, Vietnam). Fingerling pompano were acclimatized to the experimental conditions for 2 weeks by FMD feeding before the start of the feeding trial. Twenty fish with an initial body weight of 25 g each were allocated to each of the eight indoor

polyvinyl chloride tanks (500 L holding capacity), i.e. two replicate tanks per dietary treatment. The tanks were aerated and supplied with filtered seawater at a rate of 4 L/min. For 4 weeks, the fish were hand-fed the experimental diets to apparent satiation twice daily (09:00 AM and 16:00 PM). Dissolved oxygen and water temperature were monitored daily, ranging between 5.5 ppm and 6.8 ppm and between 26.2 °C to 29.4 °C, respectively.

Sampling

At the end of the feeding trial, fish were fasted for 48 hours before sampling. Blood samples were collected with heparinized syringes from the caudal veins of five fish per tank, then centrifuged (10,000 rpm for 10 min) to obtain plasma. These fish were also dissected to collect the gallbladder samples. The remaining fish was used for fecal collection, in which fish were fed the same experimental diets, and feces were collected by stripping at 4 hours after feeding. After collection of sufficient fecal matter for the determination of protein and lipid ADCs, six fish from each tank were dissected 3 hours after feeding to collect anterior and posterior intestinal digesta. The dissected fish from each tank were divided into two groups (three fish each) and the intestinal digesta in each region from each group were pooled. The division of the intestinal tract was based on descriptions by Murashita et al. (2008), and the anterior and posterior intestinal digesta were collected from the entire straight region. All samples were maintained at -20 °C until analysis.

Analytical methods

Plasma constituents were analyzed with a commercial automatic analyzer (Architect c16000, Abbott, Illinois, USA). Bile acids in freeze-dried intestinal digesta were extracted with 90% ethanol, followed by chloroform:methanol (1:1, v/v), according to the method described by Setchell et al. (1983). The extract from the digesta and bile juice diluted with distilled water at a ratio of 1:1,200 were used for total bile acid level quantification with a commercial assay kit (MAK309, Sigma-Aldrich, St. Louis, MO, USA). Lipase and trypsin in freeze-dried anterior intestinal digesta were extracted by homogenization into four and eight volumes (v/w) of cold distilled water, respectively. The homogenates were then centrifuged at 20,000 rpm for 15 min. The supernatant was further diluted 10-fold with cold distilled water. Lipase and trypsin activities were measured with the method described by Murashita et al. (2008).

To measure lipase activity, a total of 150 µl of enzyme extract was incubated with 0.4 mM *p*-nitrophenyl myristate (Sigma-Aldrich, St. Louis, MO, USA) in 24 mM ammonium bicarbonate, 7.5 mM sodium deoxycholate, and 0.5% Triton X-100, pH 8.5 (total volume: 1.5 ml). Lipase catalytic activity was then determined by measuring the rate of *p*-nitrophenol (*p*NP) production at its optimal reaction temperature (37 °C). The increase in absorbance at 405 nm was recorded every minute for 5 min. Reaction rates were calculated in units (U), such that 1 U was defined as 1 µmol of *p*NP released in 1 min. Deoxycholate were also added to emulsify lipid and activate lipase in the lipid digestion process. Therefore, lipase activity was measured under two analytical conditions, with and without external sodium deoxycholate added to the assay medium, to evaluate intestinal bile acid supply for lipase activity.

To measure trypsin activity, 54 milligrams of *N*-benzoyl-L-arginine-*p*-nitroanilide (L-BAPA, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 1 ml of dimethyl sulfoxide, and the volume was adjusted to 100 ml with Tris buffer (0.1 M, pH 11, containing 20 mM CaCl₂). The reaction mixture consisted of 1.6 ml Tris buffer (0.1 M, pH 11, containing 20 mM CaCl₂), 1 mL L-BAPA, and 100 µl enzyme extract. Trypsin catalytic activity was determined by measuring of the rate of *p*-nitroaniline (*p*NA) production at 50 °C. The increase in absorbance at 405 nm was recorded every minute for 5 min. Reaction rates were calculated in U, such that 1 U was defined as 1 µmol of *p*NA released in 1 min. The proximate compositions of the experimental diets, feces, and the digestibility marker were analyzed according to the Association of Official Analytical Chemists standard methods (AOAC, 2005). The nutrient ADC (%) was calculated as $100 \times [1 - (I_d/I_f \times N_f/N_d)]$, where I_d and I_f represent the concentrations of inert marker (chromium oxide) in the diet and feces, and N_d and N_f represent the concentrations of nutrients in the diet and feces, respectively.

Statistical analysis

Data were analyzed with one-way analysis of variance (ANOVA). Statistical differences between groups were assessed with the Tukey-Kramer test and the significance threshold was a 5% level of probability.

RESULTS

In the present study, the effects of the Ext containing alcohol-soluble components from SBM on bile acid level, digestive enzyme activity and nutrient digestibility were assessed based on plasma constituents, total bile acid level, trypsin and lipase activities, and protein and lipid ADCs.

Plasma constituents

The tested diets did not significantly affect plasma total protein and glucose levels in experimental fish (Table 2). The total cholesterol levels of SBMD-fed and ESBM+ExtD-fed fish were significantly lower than that in FMD-fed fish ($P < 0.05$), whereas there was no significant difference in total cholesterol between the ESBMD and FMD experimental groups. SBMD-fed fish had a significantly inferior triglyceride content compared to the FMD-fed group ($P < 0.05$), whereas ESBMD-fed and ESBM+ExtD-fed fish showed triglyceride levels comparable to FMD-fed fish.

Table 2. Plasma constituents of pompano fed the experimental diets¹

Parameters	Dietary groups			
	FMD	SBMD	ESBMD	ESBM+ExtD
Total protein (g/dl)	4.3 ± 0.5	4.0 ± 0.3	4.4 ± 0.4	4.2 ± 0.2
Glucose (mg/dl)	145.7 ± 12.3	135.6 ± 9.7	142.3 ± 10.4	139.8 ± 15.5
Total cholesterol (mg/dl)	296.2 ± 24.8 ^b	224.2 ± 12.7 ^a	271.3 ± 18.5 ^{ab}	232.6 ± 21.3 ^a
Triglyceride (mg/dl)	187.5 ± 14.1 ^b	146.7 ± 10.6 ^a	173.2 ± 17.2 ^{ab}	156.6 ± 12.9 ^{ab}

Notes: ¹Values are means ± standard deviations (n = 10). Values in the same row with different letters are significantly different ($P < 0.05$).

Total bile acid level

The total bile acid levels in gallbladder were significantly lower in SBMD-fed, ESBMD-fed and ESBM+ExtD-fed fish than in FMD-fed fish ($P < 0.05$) (Table 3). There were no significant differences in the biliary total bile acid levels among the SBMD, ESBMD, and ESBM+ExtD experimental groups. The total bile acid level in anterior

intestine of ESBMD-fed fish was significantly higher than those of SBMD-fed and ESBM+ExtD-fed. These three experimental groups also had significantly lower total bile acid levels in the anterior intestine than the FMD experimental group ($P < 0.05$). The experimental diets did not alter the total bile acid level in the posterior intestine among the treatments.

Table 3. Total bile acid levels in the gallbladder and intestinal digesta of pompano fed the experimental diets¹

Dietary groups	Gallbladder ²	Anterior intestinal digesta ³	Posterior intestinal digesta ³
FMD	329.5 ± 21.6 ^b	138.4 ± 17.2 ^c	48.5 ± 3.6
SBMD	244.1 ± 16.3 ^a	69.3 ± 10.7 ^a	37.8 ± 4.2
ESBMD	267.6 ± 23.5 ^a	116.4 ± 14.2 ^b	44.6 ± 6.7
ESBM+ExtD	252.5 ± 18.9 ^a	78.8 ± 9.4 ^a	39.2 ± 5.1

Notes: ¹Values are means ± standard deviations (gallbladder, n = 10; intestinal digesta, n = 4). Values in the same column with different letters are significantly different ($P < 0.05$); ²Total bile acid in the gallbladder was calculated as mmol/L; ³Total bile acid in the intestinal digesta was calculated as μmol/g dry matter.

Trypsin and lipase activities

The trypsin activity in the anterior intestinal digesta of SBMD-fed and ESBM+ExtD-fed fish was significantly lower than those FMD-fed and ESBMD-fed fish ($P < 0.05$). No significant differences in the trypsin activity were observed between FMD-fed and ESBMD-fed fish.

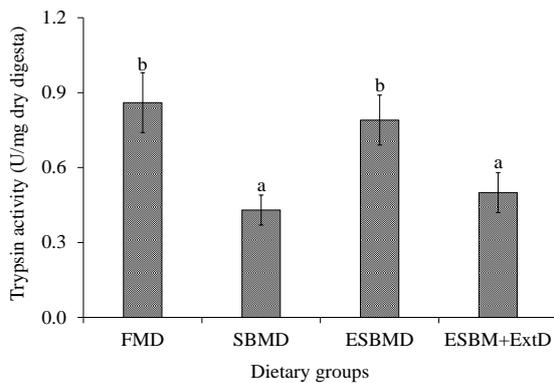


Figure 1. Trypsin activity in anterior intestinal digesta of pompano fed the experimental diets. Values are means and standard deviations ($n = 4$). Bars with different letters are significantly different ($P < 0.05$)

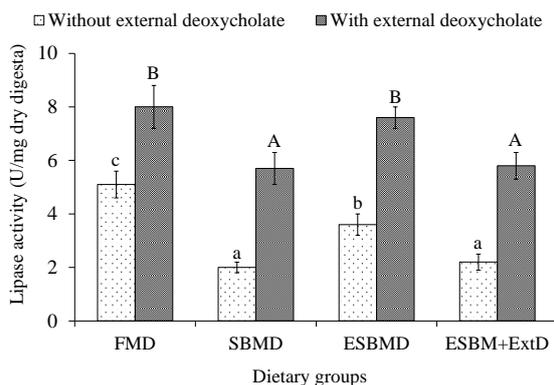


Figure 2. Lipase activity in anterior intestinal digesta of pompano fed the experimental diets. Values are means and standard deviations ($n = 4$). Bars with different letters within each analysis condition are significantly different ($P < 0.05$)

The lipase activity in the anterior intestinal digesta is shown in Fig. 2. Without external deoxycholate in the assay medium,

the lipase activity was significantly lower in the SBMD and ESBMD experimental groups than the ESBMD and FMD experimental groups ($P < 0.05$). ESBMD-fed fish had significantly lower lipase activity compared to the FMD-fed group. When external deoxycholate were supplemented to the assay medium, the lipase activity of ESBMD-fed fish was comparable to that of FMD-fed fish. these experimental groups also showed significantly higher lipase activity than the SBMD and ESBM+ExtD experimental groups ($P < 0.05$).

Protein and lipid apparent digestibility coefficients

Protein and lipid ADCs are shown in Fig. 3. Protein ADC was significantly lower in SBMD-fed and ESBM+ExtD-fed fish than the FMD-fed group ($P < 0.05$). ESBMD-fed fish had slightly higher protein ADC than the SBMD-fed and ESBM+ExtD-fed groups. No significant differences were found between the ESBMD and FMD experimental groups. The lipid ADC was significantly higher in ESBMD-fed fish than SBMD-and ESBM+ExtD-fed fish ($P < 0.05$). These three experimental group showed significantly inferior lipid ADCs compared to those fed FMD.

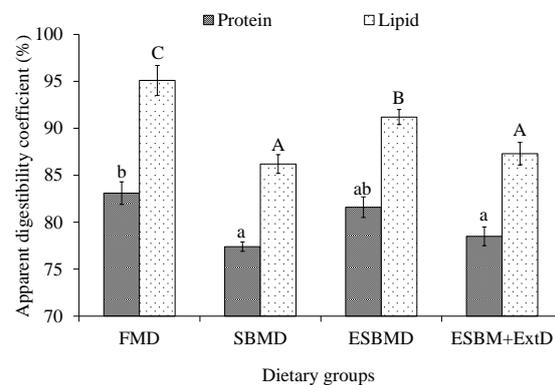


Figure 3. Apparent digestibility coefficients of lipid and protein in pompano fed the experimental diets. Values are means and standard deviations of two replicates. Bars with different letters within each nutrient digestibility are significantly different ($P < 0.05$)

DISCUSSION

In the present study, we found that the Ext containing alcohol-soluble components extracted from SBM reduced bile acid level, trypsin and lipase activities, protein and lipid ADCs, and plasma lipids concentrations in pompano fish. These negative effects of the Ext may be responsible for poor growth performance of the fish feeding on SBM-based diets.

Low biliary bile acid levels have been reported in carnivorous fish such as rainbow trout (Iwashita et al., 2008; Yamamoto et al., 2010), red sea bream (Takagi et al., 2002) and yellowtail (Goto et al., 2007; Nguyen et al., 2013) when they were fed soybean protein-based diets. Similarly, in the present study, pompano fish feeding on soybean protein-based diets (SBMD, ESBMD, and ESBM+ExtD) had significantly lower bile acid levels in the gallbladder than when FMD-fed. Bile acids are synthesized in the liver from cholesterol, then conjugated with taurine or glycine before being stored in the gallbladder (Tuchweber et al., 1996). The conjugation of bile acids in carnivorous fish is exclusive to taurine, with cholytaurine and chenodeoxycholytaurine being the main bile acids (Goto et al., 1996). It has been known that taurine availability is poor in SBM (Olli et al., 1995; Yamamoto et al., 1998). On the other hand, taurine synthesis capacity has been revealed to be low in carnivorous fish (Goto et al., 2001). Therefore, the inferior biliary bile acid level observed in SBMD-fed, ESBMD-fed, and ESBM+ExtD-fed fish compared to FMD-fed fish may be due to an insufficient supply of taurine for bile acid synthesis.

Total bile acid levels of anterior intestinal digesta of SBMD-fed and ESBM+ExtD-fed fish were significantly lower than those of the ESBMD-fed group, despite similar total bile acid levels in the gallbladder among these experimental groups. Since the bile duct connects to the anterior intestinal part (Akazaki, 1987), the lower bile acid levels in the anterior intestinal digesta of SBMD-fed and ESBM+ExtD-fed fish compared to the

ESBMD-fed group should be due to lower secretion of bile acids from the gallbladder. SBMD-fed fish showed lower total bile acid level in the anterior intestine comparable to the ESBM+ExtD-fed group. Moreover, the alcohol extract of SBM was added to the ESBM+ExtD at a ratio corresponding to its inclusion level in SBMD. These results indicated that the alcohol extract of SBM were involved in lowering the secretion of bile acids into the intestine. As alcohol extract contained mainly alcohol-soluble components extracted from SBM, this also suggested that alcohol-soluble components inhibited the secretion of bile acids into the intestine.

The activity of two pancreatic digestive enzymes, lipase and trypsin, in the anterior intestine of SBMD-fed and ESBM+ExtD-fed fish was significantly lower than that in FMD-fed and ESBMD-fed groups. Lipase and trypsin are synthesized in the pancreas, then secreted into the intestine (Einarsson & Davies, 1996). The findings of the present study indicated that the secretion and/or synthesis of lipase and trypsin were impaired by the alcohol extract of SBM. In the present study, lipase activity in anterior intestinal digesta was measured under two analytical conditions, with and without the external emulsifier, sodium deoxycholate. Under both analytical conditions, lipase activity was significantly lower in SBMD-fed and ESBM+ExtD-fed fish than in FMD-fed ones. This implies that the anterior intestines of SBMD-fed and ESBM+ExtD-fed fish were insufficiently supplied with bile acids and lipase enzyme. Lipase activity of ESBMD-fed fish was significantly lower than that of FMD-fed fish when external deoxycholate was not added to the assay medium. However, the enzyme activity of the former fish was comparable to that of the later fish by supplementing the assay medium with external deoxycholate. These results indicated that ESBMD-fed fish were insufficient in bile acids, but not lipase enzyme, which may be attributable to low bile acid synthesis caused by a poor dietary taurine content as mentioned above.

Cholecystokinin (CCK) has been known to stimulate release of exocrine pancreatic enzymes in yellowtail (Kofuji et al., 2007; Murashita et al., 2007, 2008), and also to contract the gallbladder to release bile juice in Atlantic salmon (Einarsson et al., 1997), rainbow trout (Aldman et al., 1992) and yellowtail (Murashita et al., 2007). The inhibition of secretions of bile acids and pancreatic digestive enzymes found in the present study might relate to dysfunction of CCK. Several soybean anti-nutritional factors that are soluble in alcohol have been reported to disturb the digestive physiology and intestinal morphology of fish. Soya saponin alone, or a combination of soya saponin and soya lectin caused intestinal morphological changes in rainbow trout (Iwashita et al., 2008, 2009). In addition, feeding fish with soybean molasses, which mainly contain oligosaccharides produced by alcohol extraction, reduced lipid digestibility and caused intestinal damage in the Atlantic salmon (Olli & Krogdahl, 1995; van den Ingh et al., 1996). Further studies are necessary to identify the component(s) of SBM that inhibit pancreatic digestive enzymes and bile acid secretions, as well as to clarify the mechanism of this phenomenon in pompano fish.

It has been reported that feeding fish SBM-based diets resulted in poor nutrient digestibility, especially dietary lipid (Refstie et al., 2005; Romarheim et al., 2006, 2008; Yamamoto et al., 2010; Nguyen et al., 2017; Choi et al., 2020). In the present study, both protein and lipid ADCs were significantly lower in SBMD-fed and ESBM+ExtD-fed fish compared to the FMD-fed group. In contrast, protein ADC of ESBMD-fed fish was comparable to that of FMD-fed ones. Moreover, lipid ADC was also markedly increased in the ESBMD experimental group in comparison with the SBMD and ESBM+ExtD experimental groups.

The above results suggest that removing of alcohol soluble components in SBM could increase bile acid level and digestive enzyme activities in the intestine, thus, resulting in

elevating nutrient digestibility. It has been known that bile acids are important for lipid digestion and absorption. They are not only essential for emulsification of lipids and micelle formation but are also needed for the activation of lipases (Gjellesvik et al., 1989; Romarheim et al., 2008). Hence, the insufficiency of both bile acid and lipase in the intestine should be responsible for severe impairment of lipid ADC compared to protein ADC. The severe impairment of lipid digestion and absorption might induce low plasma lipid components observed in SBMD-fed and ESBM+ExtD-fed fish.

CONCLUSION

In conclusion, alcohol extract containing alcohol-soluble components from SBM inhibited secretion of bile acids and pancreatic digestive enzymes in pompano fish, which might impair nutrient digestion and absorption. The present study suggest that removal of alcohol extract and taurine supplementation could be effective for improving nutritional quality of SBM and enhancing feed utilization and growth performance of pompano feeding on SBM-based diets.

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